DNA Synthesis Repair

Intro to DNA Repair

DNA can become damaged in many ways. The most vulnerable moment is during replication, where every single nucleotide must be copied twice (once for each template strand). Errors that occur in this process will be perpetuated for the lineage to the cell. This can lead to malignant transformation, loss of function, or death of the cell line. This is why there are so many failsafes for replication such as **RNA primers** and **DNA polymerase III's 3' exonuclease proofreading**. The 3' exonuclease's high-fidelity proofreading limits the number of errors during replication.

But errors still happen. And not only can they happen during replication, but mutations can occur from exposures such as UV radiation (**thymine dimers**), heat, or even spontaneously.

When these errors occur, mechanisms of correction must involve removing the bad nucleotide, adding a new one in its place, then sealing the link between the pentose-phosphate backbones.

When errors occur **during replication** the DNA polymerase III 3' **exonuclease** feature can be used. Exonucleases remove a nucleotide at the edge of the strand. It does so only one nucleotide at a time. A 3' exonuclease finds a nucleotide at the 3' end, with its 3-carbon position exposed and an -OH sticking out. A 5' exonuclease finds a nucleotide at the 5' end, with its 5-carbon position exposed and a phosphate sticking out. The reason exonucleases only chop off at the end of a strand, and only **one nucleotide at a time**, is because the pentose-phosphate backbone is strong, and the phosphodiester bonds are hard to break.

Endonucleases are capable of breaking that phosphodiester bond; they can remove a damaged nucleotide anywhere in the strand. However, the endonucleases aren't as exact as the exonucleases. Whenever an endonuclease cuts, it must cut in two places. The result is it usually ends up taking some good nucleotide pairings with it. This an endonuclease that can remove bad pairs **at any time in the cell cycle**, and can access **any nucleotide**, but will **remove more than just the one error**.

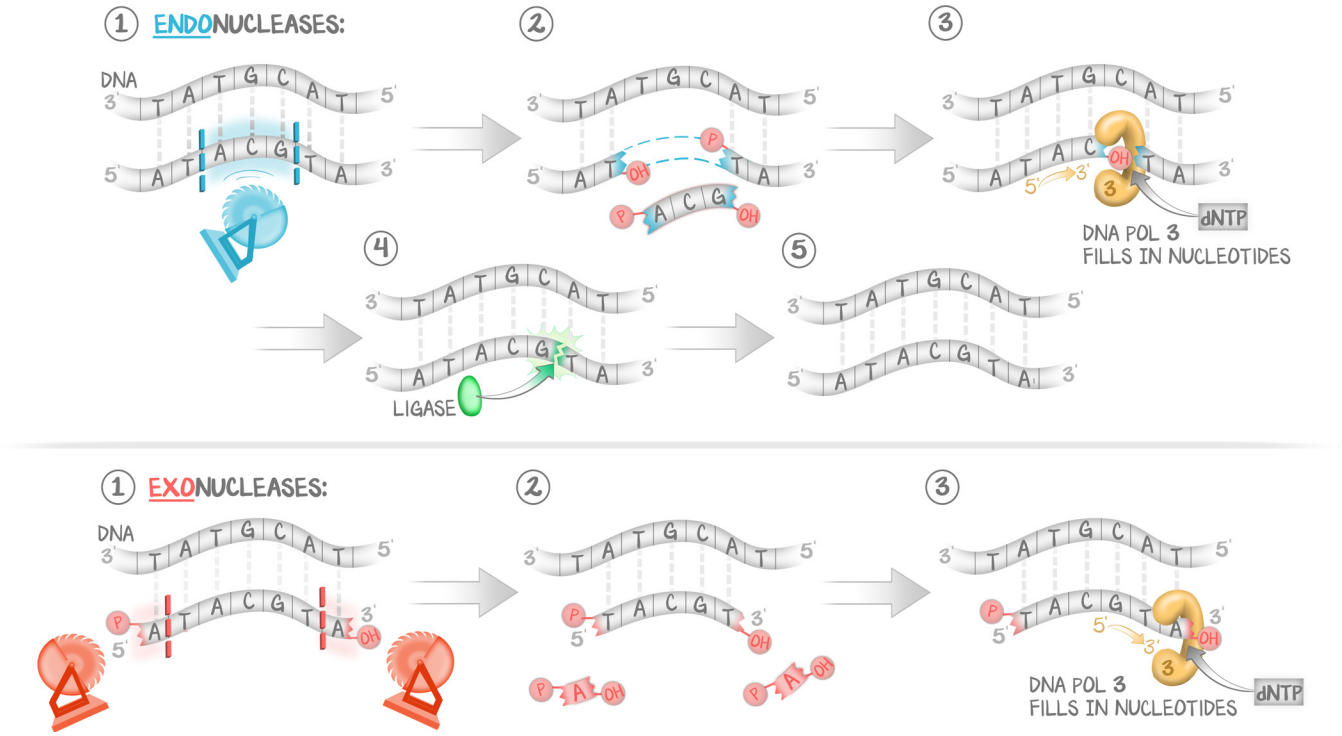

Figure 6.1: Exo- vs. Endonuclease
(Top:) Endonuclease activity removes base pairs from within the chain, without requiring an exposed 3' or 5' end, which therefore means they can take multiple at once. All strand repairs by endonucleases require ligation by DNA ligase. (Bottom:) Exonuclease activity removes a base pair from the end of the strand, requiring a free 3' or 5' end, meaning it can take only one at a time. If two strands need to be connected, a ligase can be used. But if the exonuclease activity is at the end of a growing strand, the strand could simply continue to build.

Each of the repair mechanisms discussed here utilizes **endonucleases**. The endonuclease-ing part of the repair does generally the same thing. There's some **identification** of the error, then **removal of nucleotides**, leaving behind an open segment of single-stranded DNA. The repair is completed when **DNA polymerase** fills in the gap (much as it did in replication and the Okazaki fragments) and **DNA ligase** seals the nick between the original strand and the newly repaired strand.

Thymine Dimers and Nucleotide Excisional Repair

If **two thymines** are next to each other in the same strand, they can become **covalently bonded to each other** by exposure to **UV light**. This covalent bond between the two thymines in the same strand means that there can be no hydrogen bonding to the paired adenine across the way, and it also disrupts the pentose-phosphate backbone. This disruption could cause **opening of the helix** (without stabilization from helicase or DNA gyrase), or even an abrupt **failure of transcription/replication**.

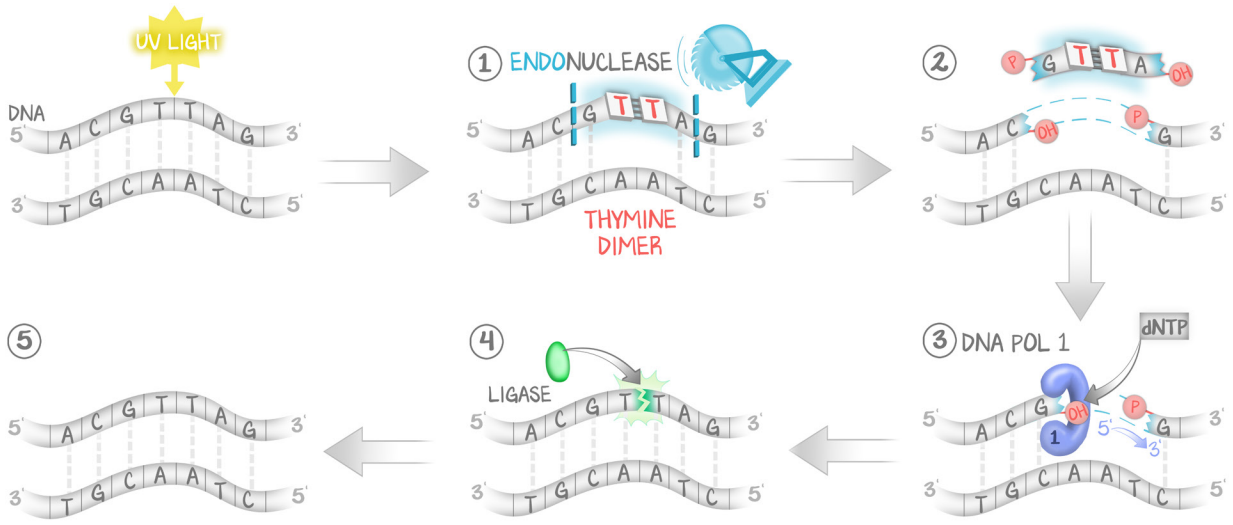

Figure 6.2: Thymine Dimers
UV light causes a thymine dimer, which is both recognized and excised by an endonuclease. DNA pol 3 fills in the gap, while ligase seals it shut.

Thymine dimers are recognized and removed by **excision endonuclease**. This one enzyme identifies the bad site, cuts the phosphodiester bonds, and pulls the nucleotides out in a single swoop. The thymine dimers are removed (along with good nucleotides), and the gap filled in by DNA polymerase and DNA ligase. The repair mechanism is nucleotide excision repair because the **entire nucleotide**—pentose, phosphate, and base—comes out.

Xeroderma pigmentosum is an autosomal recessive disorder characterized by sensitivity to sunlight, freckling, ulcerations, and skin cancer. The massive increase in skin cancer is caused by a **failure of excision repair** such that mild UV exposure produces irreparably damaging thymine dimers. It's most often due to a defect in the excision endonucleases.

Deamination of Cytosine, AP Site Exposure, and Base Excision Repair

Cytosine can be de-aminated to a uracil by either **heat** or **spontaneous error**. The uracil doesn't belong in DNA; it's a ribonucleic acid nucleotide. The error is detected by **uracil glycosylase**, which exists to identify U's in DNA. Uracil glycosylase excises the **base only**, leaving intact the pentose-phosphate backbone, now without a base where there should be one. This is called an **AP site**. That removal of the base only, but not the pentose or the phosphate, is NOT an endonuclease. But the gap where a base should be exposes the DNA strand to the next enzyme.

The second step in repair is for **AP endonuclease** to recognize the AP site and get it to a point where DNA polymerase and DNA ligase can do their work. To do that, it means identifying that the base is missing, and causing a nick in the pentose-phosphate backbone at the site of the missing base.

This is named base excision repair because the base is excised out, then a general endonuclease comes to clean it up.

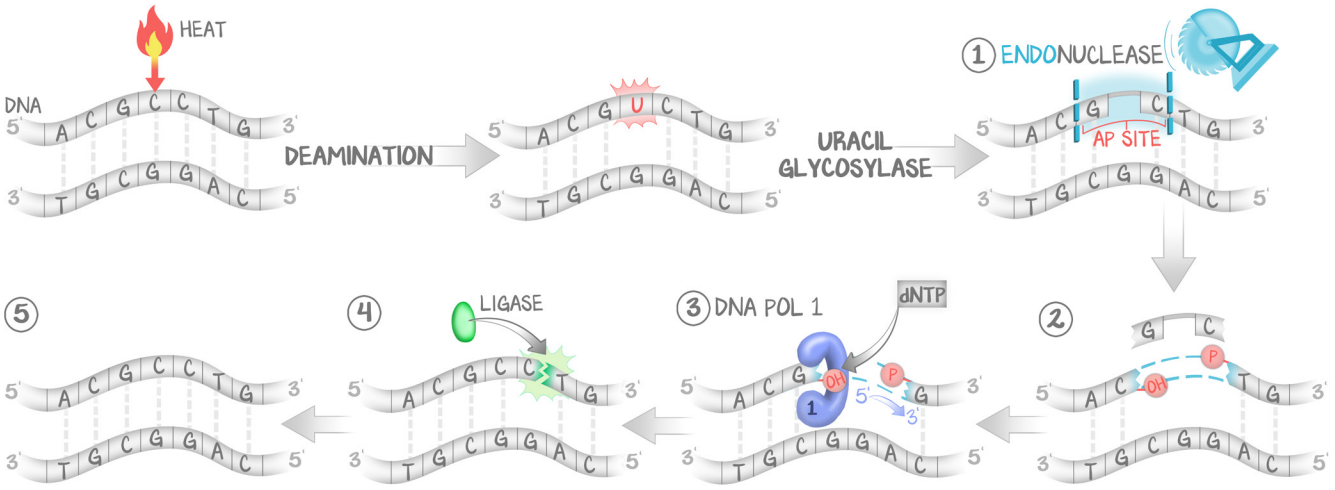

Figure 6.3: AP Site
Heat damages the DNA and induces a T to U. The uracil is recognized and the base only is removed, leaving behind an AP site. Another mechanism recognizes the AP site and removes the bad area as an endonuclease. DNA pol 3 and DNA ligase fill in the gap.

To clarify, nucleotide excisional repair results in an open fragment without nucleotides—the entire pentose, phosphate, and base are removed. It does this by immediately removing thymine dimers. Base excisional repair **ALSO** results in an open fragment without nucleotides—the entire pentose, phosphate and base are removed. It does this by first removing the base (uracil) from DNA to create an AP site, and the AP site signals the endonuclease.

Both then conclude with bridging it back together via DNA polymerase and DNA ligase.

Mismatch Repair

Before the cell progresses from G_2 into mitosis, some checks are made. DNA polymerase III is high-fidelity and corrects mistakes in replication during synthesis. But DNA polymerase III can miss an error, or spontaneous error can occur after synthesis has completed. The **mismatch repair genes** MSH2 and MLH1 are designed to double-check the DNA before the code is locked in ahead of the cell's dividing.

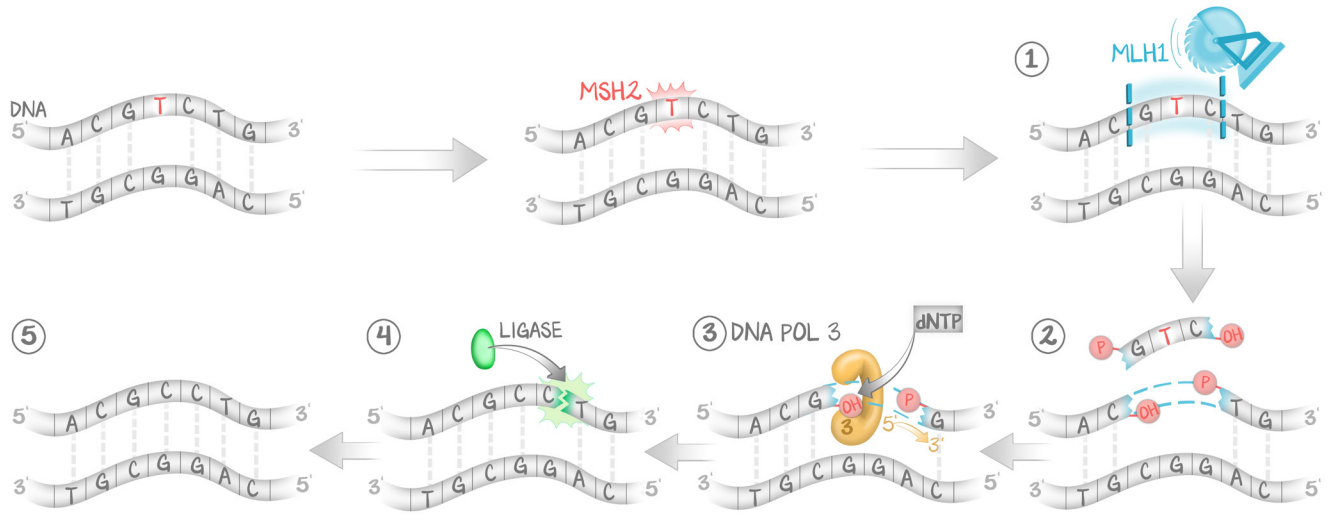

Figure 6.4: Mismatch Repair
Replication errors missed by DNA polymerase III are identified by MSH2, and removed by the endonuclease MLH1. DNA pol 3 and DNA ligase then fill in the gaps.

Mismatch repair genes have endonuclease functions and are the second check of base pair matching. During **G_2**, but before mitosis, these give the DNA a once-over. Since DNA polymerase and DNA ligase already did their thing, if they find a problem, the mismatch repair genes must be endonucleases, but do basically the same thing as DNA polymerase III's 3' exonuclease function.

MSH2 **identifies mismatched pairs**. MLH1 removes them; MLH1 is the **endonuclease**. The gap is filled in by DNA polymerase, then DNA ligase.

Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome results from the inability to repair mismatched base pairs. The introduction of mutations during proliferation greatly increases malignant transformation risk. HNPCC patients develop colorectal cancer earlier than the usual screening age and will develop colorectal cancer without polyps.